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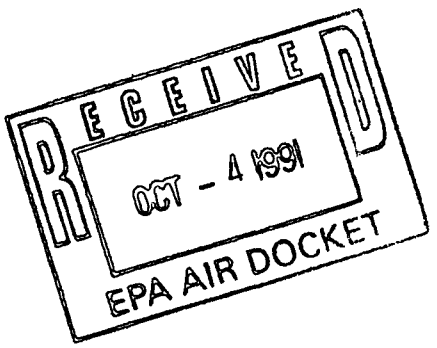

Mary T. Smith
Director
Field Operations and Support Division (EN-397F)
U.S. Environmental Protection Agency
401 M St. SW
Washington, DC 20460

RE: Docket A-91-46

Dear Ms. Smith:

I am deeply concerned about the potential public health hazard posed by increased exposure to MMT and its combustion products, and it is my considered opinion that the existing literature is inadequate to defend the introduction of this gasoline additive. I enclose the chapter that I wrote from "Air Pollution, The Automobile, and Public Health" (National Academy Press, 1988) in which I outlined the reasons for concern, the need for further review of the literature, and the need for large chronic studies in non-human primates before society seriously considers such a step.

Hazard identification cannot be characterized as complete. The existing data are inadequate to conduct a risk analysis because of the limited information about the relationship of exposure concentration and duration to effects. The risk of adverse health effects from Mn emissions should be characterized as unknown, but not necessarily unlikely. The introduction of this new air pollutant in the absence of more complete information on its pharmacokinetics and neurotoxicity would represent hubris without precedent in recent environmental regulation.

Sincerely yours,

Ronald Wood

Ronald W. Wood, Ph.D.
Research Associate Professor

Identifying Neurobehavioral Effects of Automotive Emissions and Fuel Components

RONALD W. WOOD
New York University Medical Center

Air Pollution, the Automobile, and Public Health. © 1988 by the Health Effects Institute. National Academy Press, Washington, D.C.

The automobile can be regarded as a mixed blessing. Although it has become a necessary part of daily life, the changes potentially induced by automotive emissions are not necessarily welcomed. Among these are adverse neurobehavioral effects that range in severity from the annoyance provoked by unpleasant odors and the eye irritants to overt behavioral and neurological dysfunction.

Airborne contaminants can alter behavior and the functions of the nervous system in a variety of ways. Chemicals can damage the structure of the nervous system directly, or can alter behavior and nervous system function without pathological changes by affecting neurotransmitter systems, perturbing membranes, or altering cellular metabolism. Since behavior depends upon a wide variety of nervous system functions, behavioral changes can sometimes provide early indications of adverse effects on other organ systems.

Automotive emissions can also alter behavior by stimulating sensory systems. These stimuli may be unpleasant events that alter the conduct of daily life, or may serve as important discriminative or warning stimuli.

The neurobehavioral toxicity of the chemicals involved in automotive technology is not well understood. Emissions may not produce obvious effects at concentrations commonly found in the environment; moreover, some individuals may be exposed occupationally to higher levels. Although people are exposed to the chemicals in automotive emissions environmentally as well as occupationally, and although studies of such people offer unique opportunities, it is inappropriate to rely entirely on these exposures to detect neurotoxicity, especially when suitable techniques exist using animals.

In this chapter, methods are described for the detection of adverse neurobehavioral effects of automotive emissions, and recommendations for research in this area are offered. First, ways are described of identifying, in laboratory animals, the adverse neurobehavioral effects of hazardous substances following acute exposures and repeated exposures, of characterizing subtle effects, and of determining mechanisms of toxicity.

Second, a review is provided of what is currently known about the neurobehavioral toxicity of automotive emissions and fuel constituents. Third, recommendations are offered on how to proceed with a program to address this class of health effects. Although there is reason to suspect that many emissions and fuel constituents are hazardous, little information is available for most. To address this large group of chemicals, a committee should select the substances to test and the order of testing. For the substances about which some knowledge exists, focused recommendations are offered for detailed evaluation of their hazards. These include whole emissions, carbon monoxide (CO), petroleum hydrocarbons, methanol, and metals and their compounds.

Neurobehavioral Effects and Mechanisms of Toxicity

The behavioral and neurological sciences have made extraordinary progress in the past 25 years. Although further progress will continue to yield new methods, in areas ranging from the subcellular to the behavioral levels of analysis, adequate methods are now available to explore the neurobehavioral effects of automotive emissions and fuel components.

Various approaches to neurobehavioral hazard identification have been recommended over the years by a variety of experts. Although no single comprehensive approach has yet been formalized, a responsible screening effort should include:

- identification of the acute hazards of chemicals—this includes seeking evidence of mortality, morbidity, and morphological changes (as in any acute toxicity evaluation), but with particular emphasis accorded behavioral function, learned as well as unlearned;
- characterization of their toxicity in repeated or continuous exposures—this provides an opportunity to characterize

toxicity that is delayed or cumulative, to observe the development of tolerance (or reverse tolerance), and to characterize the reversibility of adverse effects; and

- detailed study of mechanisms of injury and special impairments—this includes initial screening for subtle sensory or perceptual impairments, affective disorders, or cognitive and intellectual dysfunction, and appropriate specialized evaluations using refined neuropathological, neurochemical, and neurophysiological techniques.

The U.S. Environmental Protection Agency (EPA) has issued a series of test guidelines that are appropriate for use in acute and chronic neurobehavioral toxicity evaluation. These include guidelines for the examination of neuropathology (U.S. Environmental Protection Agency 1985c), motor activity (U.S. Environmental Protection Agency 1985b), schedule-controlled behavior (U.S. Environmental Protection Agency 1985f), and a functional observation battery (U.S. Environmental Protection Agency 1985a).

Identifying Acute Hazards

It is important to determine the acute effects of chemicals on behavior and nervous system function. Acute performance impairment can increase accident proneness and lower work efficiency; thus there can be serious consequences of even small lapses of coordination, vigilance, or visual sensitivity in operators of all types of transportation machinery. Irritation and sensory effects—perhaps the most common complaints elicited by automotive emissions—reduce the perceived quality of life, cause people to change their lifestyles (by allocating their time to less distressful activities), and incapacitate sensitive individuals. Hence, even apparently reversible adverse effects are of concern. In fact, acute but reversible effects may well be the ones of most concern (see discussion in Whole Emissions and Their Photochemical Byproducts), and information about them, therefore, is of particular importance when describing acceptable limits of exposure. With care, it is possible to design and conduct statistically valid experiments to accurately estimate the pollutant concentrations that produce small but consistent adverse effects (Wood and Colotla 1986; Wood and Cox 1986).

Acute toxicity testing requires evaluation of function and morphology, and can have outcomes that are positive or negative with respect to either. Thus the possible outcomes can be expressed in array form as:

		Morphology: No Effect	Morphology: Effect
Function	No Effect	A	B
	Effect	C	D

Morphological changes (outcomes B or D) are clearly of immediate concern. However, many chemicals can produce observable functional changes without any morphological correlates (outcome C). Lead, for example, is an automotive emission that produces functional impairments in humans and animals without marked neuropathological changes. Consequently, a complete safety evaluation requires functional tests at levels of exposure so low that they do not produce detectable morphological changes.

Examinations of conditioned behavior can be constructed to detect impairment of a variety of functions. Evaluation of function may, however, fail to warn of morphological changes in the nervous system (outcome B) until the loss of "functional reserve" is sufficiently great (outcome D). Challenges with pharmacological agents may be useful in such situations to unmask silent damage (outcome A or B).

Behavioral tests detect performance disruptions that are indirect results of effects on other systems, just as a disinclination to dance might precede the onset of diarrhea (Dews 1975). Thus motor activity or food and water consumption can be changed by chemicals that do not enter the central nervous system, and without concomitant changes in body weight (Evans et al. 1986). Irritation is another possible indirect manifestation of toxic impairment,

and is discussed further under Whole Emissions and Their Photochemical By-products. There are important gaps in our knowledge of the relationship between exposure and the acute behavioral changes it produces.

Toxicity in Repeated or Continuous Exposures

After acute toxicity has been examined, repeated exposure experiments should be undertaken, typically 28- or 90-day subchronic exposures with routine examination of neuropathology, measurement of motor activity (preferably in the home cage), and routine use of functional observational batteries. With the insights afforded by the acute toxicity experiments, repeated-exposure experiments can be tailored to further characterize those effects and to yield improved sensitivity. For example, special histological studies might be indicated; examining learned behavior in detail can provide important insights into the nature of toxic impairment (Laties and Wood 1986).

Such repeated-exposure experiments become particularly important if acute studies indicate the potential for irreversible toxicity. In such cases, for example, tolerance may play an important role, and the repeated administration of other compounds or reference substances as probes may demonstrate a forward or reverse tolerance. The mechanism of tolerance may then be revealed with an appropriate design, such as taking concurrent blood level and behavioral measurements or using satellite groups of animals for tissue-level determinations of whether tolerance results from an increase in metabolism or elimination of the toxic substance. In some cases, the mechanism of tolerance may be behavioral, and may depend only on an animal's opportunity to respond in the presence of the material. The observations of Kane and Alarie (1977) with formaldehyde and acrolein illustrate how the context of previous experience with the toxicant can affect biological response (see Conditioned Responses to Exposure).

Identifying Subtle Effects

Many sensory and perceptual deficits, affective disorders, and cognitive and intellectual dysfunctions are sufficiently subtle that they can be missed in routine acute and subchronic studies.

Sensory and Perceptual Deficits. There is a recognized need for rapid screening procedures (National Institutes of Health 1977), as well as comprehensive studies of the complex functions of sensory systems.

Vision. Toxic effects on this highly complex sensory system are easily missed, especially when studies are undertaken with rodents. For example, experiments with rodents do not reveal the profound restriction of visual fields produced by methylmercury in humans and nonhuman primates. Carbon disulfide can produce selective impairment of the discrimination of coarser features of visual stimuli, leaving the discrimination of fine features unimpaired (Merigan et al. 1985b). Carbon disulfide also affects color vision and hue discrimination (Raitta et al. 1981), an effect that can only be observed in experimental animals having color vision.

Agents that produce peripheral neuropathies (for example, hexacarbons) are likely to affect vision. Measurement of visual function in primates apparently provides a very sensitive index of central nervous system injury (Eskin et al. 1985; Merigan et al. 1985a). Appropriate studies in primates as well as rodents might support the inference of a defensible safety factor from rodent data on peripheral neuropathy. However, inferences about other compounds (for example, those with a cortical distribution of injury) are much more problematic, and reveal functional deficits only with detailed evaluation.

Audition. In most species, the inner ear is difficult to remove from the surrounding bone, so its pathologies are rarely uncovered. Lesions may be missed if specific functional evaluations are not performed. Toluene, whose toxicity has been the focus of much study for years, offers an excellent illustration. It was only recently that Pryor

and coworkers (1984a,b) and Rebert and colleagues (1983) documented a selective high-frequency hearing loss following intense toluene exposure. They discovered this damage using a pole-climb avoidance test that required animals to jump on a pole to avoid shock in response to a warning stimulus; they varied the characteristics of the tone to demonstrate the frequency-specific loss. Techniques have also been developed that do not rely on training, for example, the inhibition of noise-elicited startle by barely detectable sounds (Hoffman and Ison 1980; Young and Fechter 1983).

The loss of sensation can readily be detected using psychophysical procedures with animals, but detecting the loss of "perception" poses a more difficult problem. Some chemicals produce no impairment of hearing for pure tones, but produce profound impairment of "perceptual speed." Thus methylmercury poisoning does not alter "hearing" per se, but does impair language comprehension in such a way that individuals function only when spoken to extremely slowly.

□ **Recommendation 1.** Quick and simple tests of sensory impairment should be developed that can be used for screening in conjunction with functional observation batteries. At the same time, psychophysical procedures that are more comprehensive should be developed; these should be directed at specific sensory and perceptual impairments, for example impairment of complex auditory and visual discriminations.

Olfaction, Gustation, Somesthesis, and Proprioception. Toxic effects on these functions might be detected following high doses of chemicals, albeit with great uncertainty. Experiments could be designed to assess these end points in detail. The technologies are readily available, but knowing when best to use them can be a problem, except for chemicals that affect or react with the olfactory mucosa (and potentially the olfactory nerve and bulb), such as oxidants, aldehydes, and large particles.

Changes in olfactory sensitivity may or may not be reversible, and they can alter behavior and quality of life without the affected individual's being aware of it. People with injuries to their sense of smell complain of burning their food while cooking, or of having bouts of food poisoning from eating undetected spoiled food. These effects, as well as the techniques for studying them, have been reviewed (Wood 1982). Olfactory psychophysics is a highly developed research area in which trained observers (human or animal) are used to establish thresholds for detection of odors or for the detection of differences in intensity of odor (Cain and Moskowitz 1974; Moulton et al. 1975). Pursuing research in this area on human subjects could bear fruit. Neurophysiological techniques can detect acute alteration of nasopalatine nerve function following 1-hr exposures to formaldehyde or ozone (O_3) (Kulle and Cooper 1975).

Odor preference studies with animals are readily done but offer little predictive utility for human preferences. Subjective responses to environmental odors have been studied in humans (see, for example, Turk et al. 1974) including work on diesel odor (Springer 1974).

□ **Recommendation 2.** Studies of olfactory sensitivity following aldehyde and oxidant exposures should be undertaken in humans and rodents.

Affective Disorders. Exposure to some chemicals can produce apparently aberrant "affective" or "emotional" behavior in animals. Normally docile strains of rats exposed to inorganic mercury vapor and housed in groups have been observed to spontaneously assume aggressive postures in the home cage (Beliles et al. 1968). Inorganic mercury vapor is well known for its early production of a "neurotic" syndrome in humans. Other chemicals may exaggerate startle responses to sudden stimulation, or make animals very difficult to handle. Carbon disulfide can make dogs extremely aggressive (Lewey 1941); its effects on humans range from the induction of affective disorders to suicide (Wood 1981b). These effects, some of which can be

detected only by a careful observer, have received insufficient attention (National Institutes of Health 1977). Procedures that rely on conditioned behavior to detect affective changes produced by toxic chemicals have yet to be put to use; avoidance or punishment procedures should be well suited for this purpose.

□ **Recommendation 3.** Behavioral models of affective disorders should be developed. The models should be calibrated with reference substances, drugs as well as toxicants, before their application to test chemicals or mixtures.

Cognitive and Intellectual Dysfunction. Short of epidemiologic investigations, the only practical way to study the impairment of normal cognitive or intellectual functioning resulting from the intake of chemicals is to undertake conditioning experiments with animals (National Institutes of Health 1977; Laties 1982). Studying simple performances in well-trained animals can provide useful information, but the findings may not necessarily be associated with learning impairments. More complex performances conditioned explicitly to examine learning can provide us with insight into the likelihood of injury to this important function.

The repeated-acquisition technique has been used considerably as a model of learning impairment (Thompson and Moerschbaecher 1978). This procedure requires an animal to perform two tasks, both requiring the animal to respond in a particular sequence. For one task, the response sequence is constant from day to day, so that performance of a well-learned task is measured; for the second task, the sequence is changed daily. The animal "learns to learn" a new sequence every day, so that acquisition of a response pattern can be studied repeatedly. Toxicants and psychoactive drugs disrupt this acquisition of new response sequences. A variety of other procedures could also be used to model other aspects of intellectual functioning, including alternation procedures, conditional discriminations, and respondent conditioning procedures, to name a few.

□ **Recommendation 4.** Existing procedures should be used to examine whether toxicants of concern impair learning, memory, cognition, and intellectual functioning.

Pursuing the Mechanisms of Toxicity

The detection of toxicity in either acute or subchronic tests will generate questions that should be pursued, since the conclusive demonstration of physiological or biochemical mechanisms of action can be enormously important.

Neurochemical Evaluation. Normal behavioral activities of animals (Sparber and Tilson 1972) as well as adverse behavioral effects of exposure result in neurochemical changes. It is therefore difficult to interpret the significance of acute neurochemical changes without correlated functional observations. If large numbers of false positives are acceptable, then routine neurochemical evaluation may be desirable as a screening test; otherwise, such use of neurochemical tests is likely to be a poor allocation of resources.

Neurochemical evaluations can be very useful as adjuncts to other tests and to determine mechanisms of toxicity. Manganese (Mn) is an example where such studies could be especially useful, because Mn is likely to have specific interactions with neurochemical substrates. Furthermore, prolonged or irreversible changes in neurotransmitter (or neuromodulator) levels, turnover rates, or receptor numbers, which might be suspected following the observation of other forms of toxicity, are always important. Several good examples exist in the literature on sympathomimetic amines. Repeated amphetamine administration can produce behavioral changes and death in animals, in the absence of obvious pathology. Amphetamine-like drugs can also produce long-lasting reductions in dopamine and serotonin, and a decreased number of uptake sites in brain (Ricaurte et al. 1985). The changes found would not be obvious in a first-pass neuropathological examination. However, the destruction of some receptor populations could be demonstrated histochemically (using Fink-

Heimer silver stains), after the identification of neurochemical changes.

There probably will not be tests for neurobehavioral toxicity comparable to in vitro tests for mutagenicity (that is, the Ames test). A high rate of false positives could be expected from most such test systems; an effect in the test tube does not mean that an effect will necessarily occur in vivo, because the agent may not reach the site of action. False negatives might occur less frequently, but be of greater concern. However, nerve culture techniques will assist in clarifying the mechanisms of neurotoxicity (Veronesi et al. 1980).

On the other hand, biochemical tests can contribute to identifying particular kinds of neurotoxicity. Assays for neurotoxic esterase induction in the brain and the spinal cord have been useful for the identification of neuropathic organophosphates, and an EPA test guideline has been written for this purpose (U.S. Environmental Protection Agency 1985d). Glial fibrillary acidic protein assays reflect the astrocytic response to central nervous system injury, and may be useful as a screening technique (Brock and O'Callaghan 1987). Furthermore, biochemical assays may be able to help steer the process of chemical synthesis; thus if a test indicates possible toxicity for a chemical being developed, further synthesis work might be directed toward developing less toxic alternatives.

Neuropathological Examinations. Neuropathological examinations should be included in toxicity assessment; frequently, the same animals can be used for behavioral testing and neuropathological examination. Subsequent studies of mechanisms of toxicity, of special systems, or of chronic effects may require the study of satellite groups because immersion tissue fixation and routine staining procedures are inadequate for the description of some types of nervous system injury. For example, histochemical techniques (fluorescence, metal stains, Golgi) and immunohistochemistry can provide insights into the mechanisms underlying the neurotoxic effects of certain chemicals. Such techniques are not routinely used for hazard identification.

Special procedures are sometimes required for some classes of injuries; for example, techniques to examine axoplasmic transport. Most such techniques are incompatible with routine toxicologic evaluation. Similarly, nerve teasing and electron microscopy can demonstrate subtle neuropathies. Both are extremely labor intensive. Neuropathological studies can use special chemicals for studies of the mechanisms of toxic injury. The description of the role of γ diketones in the production of hexacarbon neuropathies offers a good example; subsequent studies of 3,4-dimethyl-2,5-hexanedione have examined accelerated pyrrole formation and its role in potential neurofilament cross-linking (Anthony et al. 1983a,b).

Neurophysiological Examinations. Peripheral nerve conduction velocity is used clinically to assess peripheral nerve function in humans. In animals, hind limb weakness, gait disturbance, and peripheral neuropathology provide adequate sensitivity for hazard identification, given that the exposure concentrations contemplated are high enough to induce frank peripheral neuropathy. Measurement of peripheral nerve conduction velocity may be useful for the detection of demyelination, and the U.S. Environmental Protection Agency (1985e) has promulgated a test standard for this purpose. However, it may not detect axonopathies. Most important, the reversibility of peripheral nerve impairments need not imply the reversibility of central nervous system injury.

Evoked-potential studies might be cost-effective for some classes of sensory effects, especially to determine low-level effects. Brainstem auditory evoked responses are useful in detecting hearing loss; the sensitivity of these procedures is comparable to that for behavioral procedures (Rebert et al. 1983). Flash-evoked responses are less informative about injuries to the visual system than are counterphase spatial-frequency reversal experiments. More advanced procedures are available to characterize the interplay of structural and functional alterations produced by toxicants. By simultaneous measurement throughout a brain structure, it

should be possible to describe the pathophysiological progression of injury.

Evaluating Whole Emissions, Fuels, and Their Components

Although it is possible to study whole emissions directly as complex mixtures, this is usually not the most fruitful course to pursue. The difficulties encountered in studying such complex mixtures are profound, because the myriad interactions possible among the reactive components of automotive emissions may result simultaneously in potentiation of, and protection against, adverse effects. The study of mixtures should not preclude the continued evaluation of the toxicity of individual components of mixtures, especially since the reduction or elimination of a single component could have major health impacts. We learn relatively little from the study of a single idiosyncratic mixture or simulated automotive emission; indeed, in such studies, the data collected on the reference substance used to calibrate the sensitivity of the experiment may well constitute a greater scientific contribution than the data from the mixture (Laties 1973; Horvath and Frantik 1974).

More information about the behavioral effects of carcinogenic emissions is needed. When testing for carcinogenesis, pollutant doses and concentrations are usually maximum tolerated doses; those that typically produce behavioral effects are often much lower. Thus, under ambient conditions of exposure, the behavioral, rather than the carcinogenic, effects of carcinogenic components of emissions may be the principal effects of concern; these effects might include malaise or performance degradation.

Whole Emissions and Their Photochemical By-products

Air pollution episodes alter human behavior. Weather reports in certain metropolitan areas regularly include air quality reports, and the elderly, those with respiratory problems, and athletes modify their behavior accordingly. The description of atmospheric conditions (even an erroneous prediction) may change the activities people engage in; for example, in a form of conditioned avoidance behavior, the heightened probability of chest discomfort in a smog alert may lead an athlete to change his or her training regimen. During the 1984 Olympics in Los Angeles, concern about the potential effects of automotive emissions and photochemical products on performance and health prompted recommendations for the siting of athletic competitions and for traffic control, as well as for training and competition schedules (McCafferty 1981).

Even if individuals cannot articulate the association between verbal warnings about air quality and discomfort during exercise, they may nevertheless avoid the circumstances in which unpleasant sensations occur, without directly attributing their avoidance to atmospheric quality. The extent to which unpleasant or uncomfortable sensations alter the behavior patterns of daily life has not been recognized.

Nervous system function can be directly affected by constituents of whole emissions, such as CO. Emissions can also produce effects mediated by less direct mechanisms. Functional disturbances that occur in response to emissions may be conditionable in and of themselves, as one might condition a dog to salivate following the ringing of a bell; the evidence for such conditioning will be discussed later.

Eye Irritation. Eye irritation is the most frequent complaint evoked by emission exposure. Numerous studies have documented the increasing frequency of complaints with increasing contaminant concentrations. In a report on O_3 and other photochemical oxidants, the National Academy of Sciences (1977a, p. 430) asserted "For the two most prevalent symptoms related to photochemical-oxidant exposure—eye irritation and lacrimation—no method of quantification has been developed. Eye irritation, although real, is a subjective response of the subject, and no measurement, other than the complaint it-

self, has yet been developed. . . ." In another report on pollutants, the National Academy of Sciences (1976) stated that the first uncomfortable reactions are usually felt in the eye tissues. That report examined several studies of eye irritation, including one by Schuck and collaborators (1966). Those investigators conducted eye-only exposures, and permitted subjects to turn a knob that adjusted the position of a pointer on a scale to indicate the eye irritation intensity experienced at any instant during the 5-min exposure. Concurrently, the rate of eye blinking was measured. Orderly functional relationships to exposure emerged from this study, demonstrating the value of such experiments. Comparable studies were undertaken more recently with formaldehyde and sidestream cigarette smoke (Weber-Tschopp et al. 1977).

Studies are most frequently performed by having questionnaires completed by members of exposed populations (for example, Heuss and Glasson 1968; Hagberg et al. 1985), or by exposing panelists to test atmospheres, measuring the elapsed time to a complaint, and eliciting a subjective rating (Bender et al. 1983). Such scaling studies could profit from better utilization of psychophysical scaling procedures developed for the study of odorants (Cain and Moskowitz 1974).

The development of procedures to assay eye irritancy in animals would be desirable. In vitro tests designed to replace the Draize test (instillation of chemicals into the eye of a rabbit) are currently being validated, but neither in vitro tests nor the Draize test can directly address the subjective response to low-level sensory irritation. Blink rate measurements are useful, but with repeated experience in the test situation, animals may learn to close their eyes to avoid exposure. Procedures that permit animals to terminate but not to avoid graded concentrations of irritants are discussed in some detail below. These latter procedures give the animal the opportunity to control the unpleasant stimulation, unlike techniques that require measurement of structural changes following instillation of the material into the eye. Another potential advantage of these procedures is that the cornea is served by the trigeminal nerve, and stimulation of this nerve decreases respiratory and heart rate. These physiological changes are of interest per se and may yield sensitivity comparable to behavioral measurements without training the subject. In any circumstance, the sensitivity of these tissues to irritants may permit the identification of the threshold for aversive stimulation.

□ **Recommendation 5.** Quantitative procedures should be developed using eye-only exposures to provide estimates of aversiveness derived from behavior under the control of irritant stimulation, and from measuring blink, heart, and respiratory rates.

Behavioral Effects. *Wheel Running.* Several experiments describing the effects of whole emissions on animal behavior provide leads for further work. Boche and Quilligan (1960) accustomed mice to running wheels, and then put one in a chamber with filtered air, and another in a smog-like mixture of O_3 and gasoline. The mice were moved from one chamber to the other every day (three 1-day exposures in each chamber), permitted to rest for a week, and then subjected to the same process at a higher smog concentration (figure 1). Although there was no assessment of running in the exposure chamber in the absence of smog, a concentration-related reduction in running was observed. This set the stage for a series of experiments demonstrating the utility of behavior for the study of emissions and photochemical products.

Gage (1979) exposed mice in running wheels to emissions from an automobile engine burning unleaded gasoline. The exhaust from the engine under lean tuning suppressed running in proportion to exposure concentration. The exhaust of an optimally tuned engine had no effect. Ultraviolet irradiation of the exhaust simulated photochemical smog, which suppressed running to a greater extent than nonirradiated exhaust. Activity returned to normal several days after the termination of irradiated exhaust exposure. However, termina-

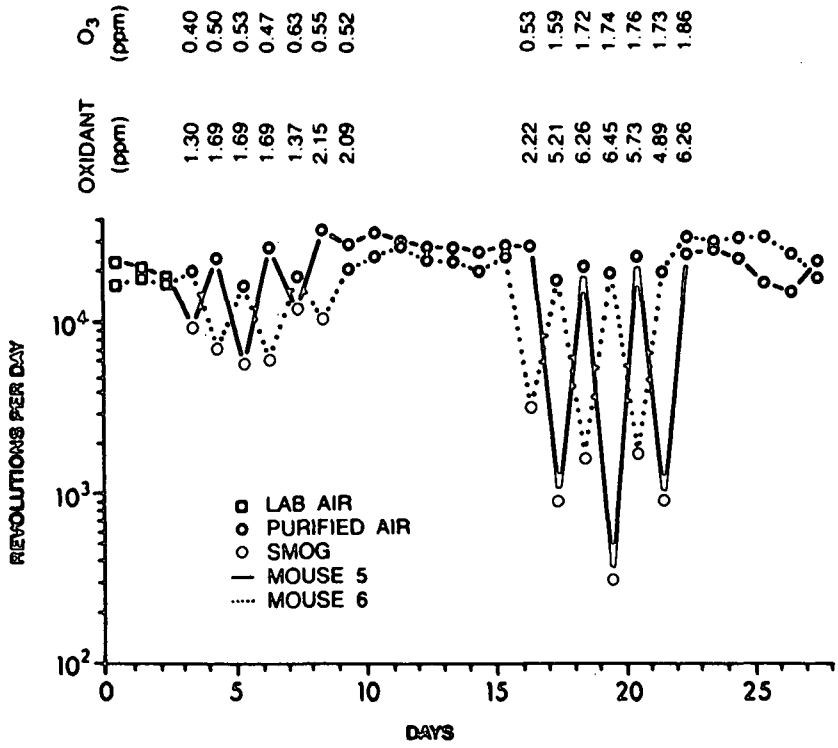

Figure 1. Spontaneous wheel-turning activity of two C57 black male mice in different environments. The total oxidant and O_3 determinations are shown at the top of the graph for each day of exposure to synthetic air pollutant mixture of O_3 and gasoline. (Adapted from Boche and Quilligan 1960.)

tion of nonirradiated exhaust produced rebound hyperactivity (figure 2, upper right panel). This study was not the first report of exhaust-induced hyperactivity (Hueter et al. 1966; Emik and Plata 1969; Stinson and Loosli 1979). These studies could have been improved by more complete reporting of the effects across time, and of their relationship to concentration.

Several investigators have used wheel running to examine biological effects of smog constituents (see, for example, the review by Murphy 1964). Murphy and colleagues (1964) observed a 46 percent reduction in activity following a 6-hr exposure to 0.2 ppm O_3, and a 20 percent reduction following exposure to 7.7 ppm nitrogen dioxide (NO_2). Campbell and coworkers (1970) demonstrated that peroxyacetyl nitrate, a constituent of photochemical air pollution, depressed running in proportion to the exposure concentration. Emik and Plata (1969) and Emik et al. (1971) set mice in wheel-running cages between the lanes of the Hollywood freeway and demonstrated an association between oxidant concentration and depressed running.

Because humans complain following exercise in O_3, Tepper and colleagues (1982) undertook a detailed analysis of the temporal patterning of wheel-running behavior that revealed disruption during 6 hr of exposure to 0.12 ppm O_3, the current ambient air quality standard. Low concentrations increased the duration and number of pauses, but did not change the speed at which the animal ran, or the length of an individual running burst. Unlike the effects on learned behavior described below (Weiss et al. 1981), effects were obvious in the first hour at lower concentrations, and performances declined throughout exposure.

Thus low concentrations of automotive emissions or photochemical reaction products can impair wheel-running performance. The mechanism by which these changes are produced should be a focus of the research agenda because it is of interest per se and because it contributes to risk analysis.

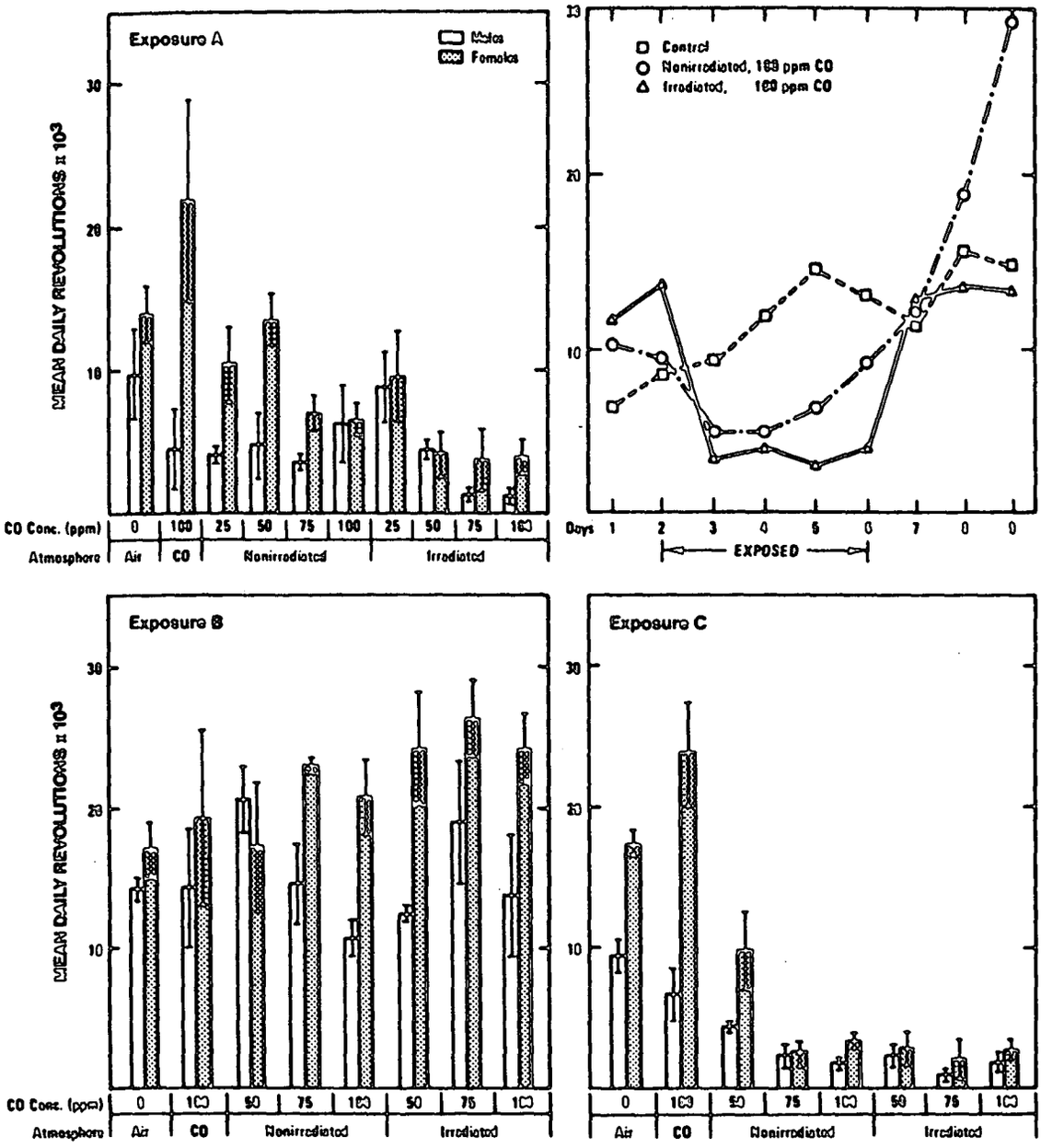

Figure 2. Mean daily activity of mice groups during emission exposure periods of the three exposure tests. Exhaust level is described by the nominal CO concentration. During exposures A and C, engine was tuned to factory specifications. During B, engine was optimally tuned. Upper right panel shows activity over the course of exposure A for male and female mice exposed to concentrations indicated. (Adapted with permission from Gage 1979.)

Mechanistic Experiments. Several mechanistic experiments indicate that the suppression of wheel running performance was probably not due to acute aversiveness of O_3. Wood (1979, 1981a) developed a procedure that permits direct behavioral assessment of the aversive properties of inhaled materials. Mice poked their noses into a conical recess to terminate the delivery of an irritant, and simultaneously produced a facial shower of clean air. Tepper and Wood (1985) demonstrated that O_3 reliably maintained escape behavior, and that its aversive properties were not dependent on previous experience with irritants. Performance was related to concentration,

and escape behavior was maintained at concentrations above 0.5 ppm. However, the concentration for the mouse that produces observable reductions in running lies between 0.2 and 0.5 ppm (Murphy et al. 1964; Tepper et al. 1985), and since running was not immediately reduced at 0.2 ppm, acute aversiveness is probably not the principal mechanism responsible for performance degradation.

Furthermore, the effects on running produced by O_3, a typical lower-airway irritant (Alarie 1973), differed from those produced by typical upper-airway irritants. Ammonia immediately reduced running; at the lowest concentration (100 ppm), mice displayed a transient increase in running, followed by a large decrease that was immediately reversible after exposure termination. In contrast, O_3 produced delayed decreases in wheel running that were sustained after the termination of exposure. Although the direction of the effect was the same for the two irritants, the time course of the effects and recovery differed dramatically (Tepper et al. 1985).

Although mice and rats responded similarly and in the same order of potency to O_3 and ammonia, rats were more sensitive, as measured by associated morbidity, to both these irritants. At first glance, this seems to contradict other investigations of sensory irritants: mice display decreased respiratory rate to upper-airway irritants at lower concentrations than rats (Chang et al. 1981). Formaldehyde offers a striking example; since decreased respiratory rate minimizes the delivered dose of formaldehyde, the species difference could account for the selective induction of nasal carcinoma in rats (Swenberg et al. 1980; Albert et al. 1982). Comparable mechanisms may be at work in wheel running in the presence of O_3 as well; rats may display greater reductions in wheel running than mice because they receive a larger effective dose (Tepper et al. 1985).

Exertion appears to be the predominant determinant of sensitivity. Weiss and colleagues (1981), interested in O_3 effects on learned behavior, conditioned rats to press a lever that produced a food pellet; 5 min later, another food pellet was available contingent upon a response. This procedure is called a fixed-interval (FI) schedule of reinforcement (reinforcement is the process by which behavior is maintained in frequency by its consequences). This relatively sedentary schedule generated response rates that gradually increased until food delivery. Rats were exposed to O_3 once a week for 6 hr, thereby decreasing their response rates late in the exposure. Increased concentrations of O_3 caused lever-pressing rates to decrease earlier in the session, resulting in reduced total output. Although effects occurred at 0.71 ppm, this performance was not as sensitive to O_3 concentration as wheel running performance was (Tepper et al. 1982). Tepper and Weiss (1986) also demonstrated comparable insensitivity with another sedentary learned performance, stochastic reinforcement of waiting (SRW), shown in figure 3. But when conditioned wheel running was used, instead of conditioned lever pressing, sensitivity was comparable to that of free-access wheel running. Thus if physical effort was required by the experimental animal, the sensitivity of the assay increased, regardless of whether the performance was conditioned.

Ozone produced specific "motivational" effects. Wheel running maintained by food reward was sensitive; sedentary conditioned behavior maintained by food was insensitive. Thus the effects of O_3 at low concentrations were not dependent on the motivational properties of food. Ozone between 0.2 and 0.5 ppm did not affect lever pressing maintained by food, but did reduce comparable performance maintained by the opportunity to exercise (fixed ratio lever press, figure 3). Compared to lever pressing for food, lever pressing for wheel running was much more sensitive; thus the effects of O_3 cannot be attributed to an effect on lever-pressing behavior per se.

The most important implication of these mechanistic experiments is that O_3 at low concentrations reduces the rewarding properties of exercise. Techniques are available to study this explicitly (for example, Pierce et al. 1986).

Effects of Repeated Exposure. Chronic exposure experiments with animals have raised several concerns. Many of the acute

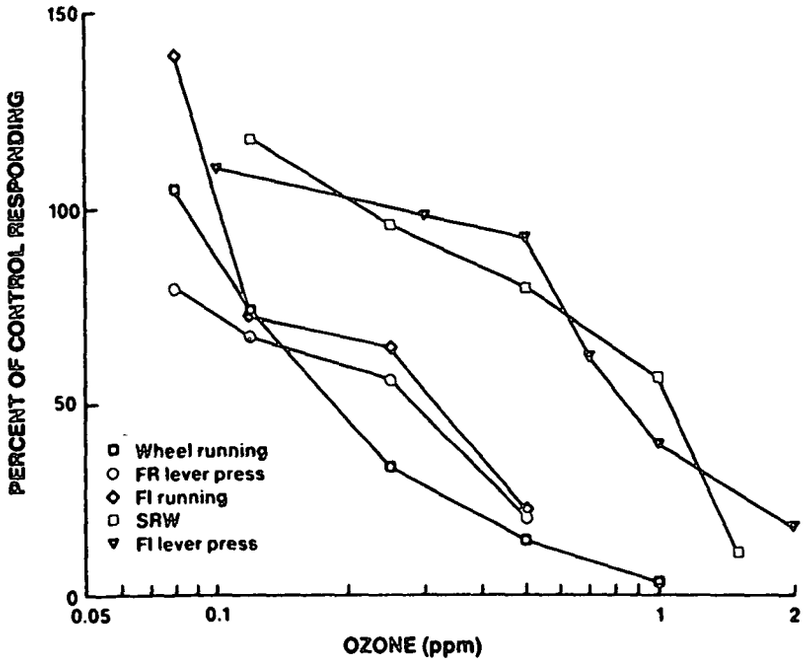

Figure 3. A summary of five behavioral experiments. The fixed-interval lever press (▽) and SRW experiments examined the effects of O_3 on sedentary conditioned behavior maintained by food, and were relatively insensitive. Wheel running was more sensitive (▣); wheel running for food reward (FI running, ◇) was comparably sensitive. Pressing a lever to produce the opportunity to run for 15 sec was comparably sensitive (FR lever press, ○). Effects of O_3 at low concentrations were not a function of effects on the motivational properties of food, were exacerbated by exercise, and reduced the rewarding properties of exercise. O_3 between 0.2 and 0.5 ppm did not reduce performance maintained by food, but did reduce comparable performance maintained by the opportunity to exercise. (Adapted with permission from Tepper and Weiss 1986.)

manifestations of exposure described above wane in intensity with repeated exposure. This response could be interpreted as simple adaptation to exposure or habituation to aversive stimulation. However, Hueter and coworkers (1966) demonstrated that tolerance to automotive exhaust was followed by delayed degradation of performance. In a similar experiment (Stupfel et al. 1973), rats exposed to exhaust gases 8 hr/day, 5 days/week for 24 months were slower to learn to run across the box to a shock-free area when a tone warned of impending shock; it has not been determined whether the rats' ability to learn, or their ability to perform the task being used to assess learning, was impaired, although procedures are available to differentiate such effects. These findings suggest that the waning of responsiveness to acutely effective materials might bear some predictive relation to delayed toxicity, perhaps via the loss of protective mechanisms.

□ **Recommendation 6.** Several types of behavioral experimentation are needed to characterize the active agents in whole emissions and photochemical by-products. Acute studies should elucidate the mechanisms of the effects by comparing automotive emissions with agents that have well-described effects on the respiratory system. Repeated exposures should reveal the agents responsible for rebound hyperactivity. Mechanistic studies should focus on behavioral determinants of sensitivity and associated changes in irritant receptors in the upper airways, lung or lung innervation, frank lung injury, and alterations in the peripheral or central nervous systems.

□ **Recommendation 7.** An animal model of compromised pulmonary function should be developed, because compromised humans are particularly sensitive to oxidant exposure. Experimental models of chronic obstructive pulmonary disease

would permit quantitative evaluation of exaggerated oxidant sensitivity and might display behavioral effects that resemble those produced by chronic exposure to oxidants.

Conditioned Responses to Exposure. It is well known that an aversion to a favorite food can develop through association of that food with an illness. There is reason to expect that disruptions of function that occur in response to emissions may be equally conditionable.

Acute exposure to sensory irritants produces a reduction in respiratory rate. Kane and Alarie (1977) studied the effects of single and repeated exposures to formaldehyde and acrolein. In one experiment, a large number of animals were assigned to either an exposure or a control group. The animals in the exposure group underwent repeated irritant exposures in an exposure chamber (lower panels, figure 4), but the animals in the control group were exposed to air. Animals in both groups were then placed in a plethysmograph and exposed to the irritant once only, at a single test concentration. Although a range of test concentrations was used, no animal was subjected to more than one such test exposure. The animals preexposed to formaldehyde did not show altered sensitivity to its acute effects; the animals preexposed to acrolein appeared to exhibit some tolerance—a lessening of response—to acrolein following repeated exposure. But, in the second experiment (top two rows of panels, figure 4), the animals were repeatedly exposed in the plethysmograph; subsequent exposures produced large, prompt respiratory rate reductions that became more pronounced with repeated exposure. The magnitude of the effect was related to concentration, and occurred for both acrolein and formaldehyde. Similarly, in our own work on irritant escape, we have observed progressively greater sensitivity to both O_3 (Tepper and Wood 1985) and formaldehyde (Wood and Coleman 1984). Repeated exposure in the same context may produce conditioned hypersensitivity to airborne contaminants reflected in changes in respiratory function.

There are other examples where airborne irritants function as unconditioned stimuli that can condition responses to occur to previously neutral cues. Alarie (1966) demonstrated the conditionability of respiratory rate decreases in his early work. Hoffman and Fitzgerald (1978) conditioned heart rate and blood pressure using ammonia. Jamison (1951) measured hearing by conditioning ammonia-induced bradycardia to occur following the presentation of a previously neutral tone.

Animal models have also been used to examine the conditioning of allergic responses in animals. Asthmatic responses have been conditioned in guinea pigs (Noelpp and Noelpp-Eschenhagen 1951a,b,c; Ottenberg et al. 1958; Justesen et al. 1970). Russell and colleagues (1984) conditioned histamine release to occur following the presentation of an odor by pairing the odor with a substance to which the guinea pigs had been immunologically sensitized.

These efforts bear broad implications for other potentially conditionable signs and symptoms, ranging from asthmatic attacks to complaints of breathing difficulties. Concentrations that were once ineffective for the elicitation of complaints or behavioral disruption can become effective through respondent (Pavlovian) conditioning mechanisms. If, however, exposure levels are held below those that initially produce the unconditional response, conditioning does not occur.

□ **Recommendation 8.** Conditioning experiments should study the range of effective concentrations and the temporal parameters that are necessary for automotive emissions to cause conditioned hypersensitivity. Threshold concentrations for the appearance of signs and symptoms of toxicity, ranging from asthmatic attacks to complaints of breathing difficulties, could then be determined.

Carbon Monoxide

The neurobehavioral effects of CO have been intensively studied (Laties and Merigan 1979). Most effects on behavior generated by exposure to low concentrations of

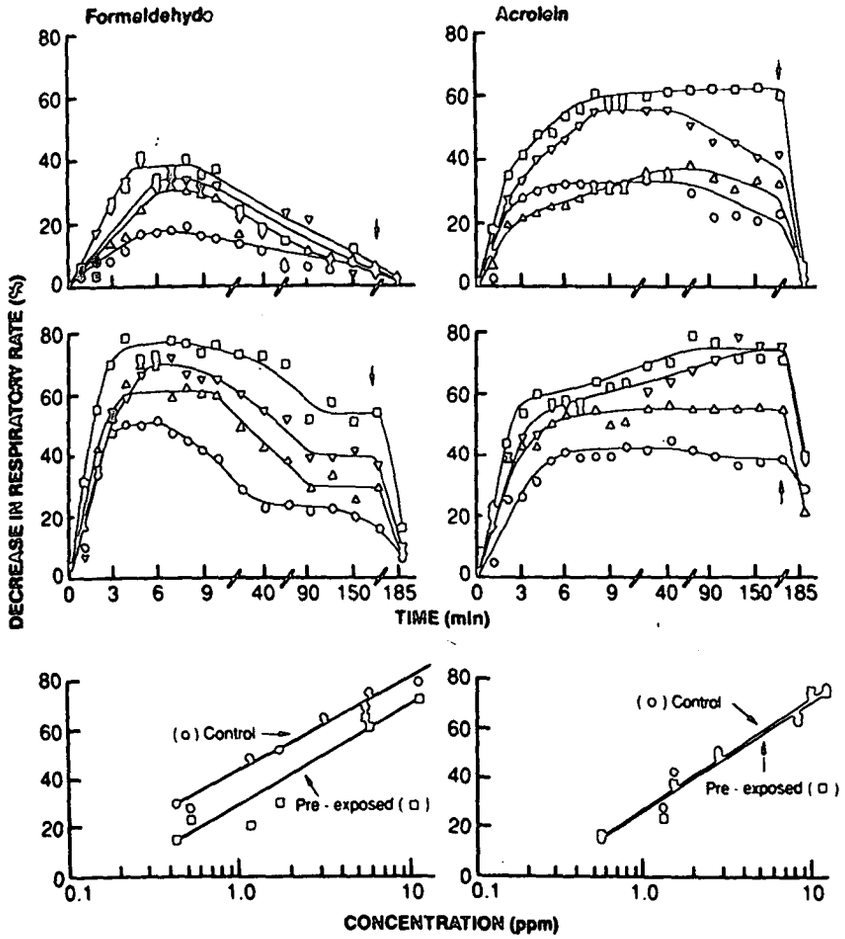

Figure 4. Progressively increasing sensitivity to formaldehyde and acrolein with repeated exposure. The time course of respiratory depression was studied during repeated exposure to two concentrations of formaldehyde (1.0 ppm, top left; 3.1 ppm, middle left) or acrolein (0.5 ppm, top right; 1.7 ppm middle right). The first, second, third, and fourth days of exposure are designated by O, Δ, ∇, and □, respectively. Arrows indicate the end of exposure periods. The bottom panels depict concentration/effect relationships for formaldehyde (left) and acrolein (right) for control mice, and for mice previously exposed in a different context to either 0.31 ppm formaldehyde or to 0.17 ppm acrolein for 3 hr/day for 3 consecutive days. No sensitization is apparent for these mice, suggesting that the increased responsiveness observed in the upper two panels was a conditioning phenomenon dependent on the context of exposure. (Adapted with permission from Kane and Alarie 1977.)

CO appear to be marginal; nonetheless, even small effects can have serious consequences for the operators of heavy machinery. Furthermore, the effects of CO depend on the behavior under study: one behavior may be disrupted while another is unimpaired, and the impairment may depend on the assessment procedures. Adequate studies of behavioral impairment need to examine highly reliable effects studied intensively in individual subjects, using multiple exposure concentrations. This is especially important since concentration/effect and time/effect functions may not be monotonic. This is an area that could profit from further experimental work with humans.

Additional effort should be made to examine teratological effects, since prenatal exposure to CO can alter the behavioral performance of offspring (see, for example, Fechter and Annau 1977; Mactutus and Fechter 1984, 1985; Storm and Fechter 1985a,b). This class of effects has received inadequate attention.

The following research recommendations are derived from a comprehensive critical review of the behavioral effects of CO (Laties and Merigan 1979), from the recommendations of the Clean Air Scientific Advisory Committee of the EPA Science Advisory Board (letter from M. Lippmann to W. Ruckelshaus, December 30, 1983), and from the report of a task force on research planning (National Institutes of Health 1977).

□ **Recommendation 9.** Systematic studies should be performed to determine which aspects of performance are most susceptible to disruption by CO.

□ **Recommendation 10.** Studies should be performed to determine which parameters of CO exposure are most important in producing behavioral impairment. Furthermore, since operators of motor vehicles are frequently under the influence of prescription and over-the-counter drugs, as well as drugs of abuse, the interaction of these substances with CO should be described.

□ **Recommendation 11.** Since the elderly and those with cardiovascular or respiratory insufficiency may display exaggerated sensitivity to CO, studies of behavioral effects with these groups should be undertaken.

□ **Recommendation 12.** Experiments utilizing the techniques of modern developmental neurobiology should be undertaken to further elucidate the effects of pre- and perinatal CO exposure.

Petroleum Hydrocarbons

Aromatic and aliphatic hydrocarbons and alcohols all can produce acute performance impairment; the more volatile and lipophilic the fuel, the more likely that such impairments could result following inhalation. Petroleum hydrocarbons display a variety of effects: some may resemble central nervous system depressants in their ability to increase the frequency of behavior suppressed by punishment, in their anticonvulsant effects (Wood et al. 1984), and in their discriminative stimulus properties (Rees et al. 1986). Others may be frankly convulsant or proconvulsant; alkylcycloparaffins offer a good example (Lazerew 1929; Lazerew and Kramneva 1930; Pryor et al. 1978). Interactions can occur that can mask or potentiate such effects; Pryor and coworkers (1978) demonstrated that the expression of overt methylcyclohexane seizures was blocked when it was administered in a mixture of heptanes. Some hydrocarbons are neuropathic—for example, *n*-hexane (Spencer et al. 1980). The production of peripheral neuropathies can be potentiated by components of the mixture; in fact, the toxicity of *n*-hexane is most evident when it is coadministered with other solvents.

The Health Effects Institute (1985) report on Gasoline Vapor Exposure and Human Cancer recognized the potential induction of neurotoxicity. That report recommends the evaluation of neurotoxicity in future chronic and subchronic animal studies. The clinical and animal literature is sparse, except for the large body of literature on the deliberate inhalation of leaded fuels. However, several Scandinavian countries have identified a new disease entity called the solvent syndrome, which has been linked to complex solvent mixtures, components of which are also automotive fuel constituents.

Although controversy surrounds the studies cited to document the syndrome's existence (Grasso et al. 1984; Errebo-Knudsen and Olsen 1986), it is well enough accepted to have become a compensable injury in several of these countries (Flodin et al. 1984). In these studies, painters were exposed for long periods to organic solvent vapors (Arlien-Soborg et al. 1979; Lindstrom et al. 1984). Solvent abusers, on the other hand, expose themselves to extremely high concentrations and experience toxicity more promptly. But both groups display similar impairments: reports of neurological examinations and computerized axial tomography provide evidence of brain atrophy in both groups (Bruhn et al. 1981; Schikler et al. 1982; Fornazzari et al. 1983; Lazar et al. 1983). These changes

occur without striking localized lesions, and are best revealed with detailed measurements of brain dimensions (morphometry) (for example, Rodier and Reynolds 1977; Rodier and Gramann 1979; Fornazzari et al. 1983). Recent claims that this syndrome occurs at or below current recommended occupational exposure limits raises concerns about allowable exposure. A recent Consensus Conference recommended directions that could be adopted for human as well as animal research efforts (Baker et al. 1985). A primate model of the solvent syndrome should be developed. Repeated acquisition procedures would be an appropriate baseline for the study of learning impairment (Thompson and Moerschbaecher 1978; Dietz et al. 1979; Howard and Pollard 1983). Repeated acquisition procedures are dependent on intact memory function. Procedures for the explicit evaluation of impairment of remembering are available (Heise 1975; Bartus 1979; Taylor and Evans 1985).

Although it is less likely that profound nervous system injury will occur at environmental levels of exposure, the likelihood of injury for fuel handlers and consumers is unknown. The neurotoxic hazards of gasoline and its constituent fractions are poorly characterized; neurotoxicologic investigations thus far have failed to use the appropriate protocols in examining the hazards. As discussed earlier, selecting a strategy for establishing priorities is a complex issue. Thus, to document the hazard posed by a particular fuel, it is necessary to study a complete complex mixture. However, fractions could perhaps be examined by class to identify hazards, an approach that might be well suited to petroleum-refining technology as well. But examination of representative constituents—that is, single chemical entities—provides the most useful information to the neurotoxicologist. Therefore, a combination of these approaches is undoubtedly best. And since the problem of potential nervous system injuries resulting from exposure to hydrocarbons is not restricted to the motor vehicle and allied industries, it may be possible to examine it in a coordinated interindustry effort.

□ **Recommendation 13.** Quantitative brain morphometry should be performed following prolonged exposure to automotive fuels. This is necessary because chronic solvent exposure does not usually produce gross lesions, but rather a selective wasting syndrome that may not be evident in measurements of gross brain weight.

□ **Recommendation 14.** Acute effect studies would help determine if the prevention of acute effects of petroleum hydrocarbons is sufficient to prevent chronic toxicity. Acute performance impairment should be a major focus of effort.

□ **Recommendation 15.** A primate model of the solvent syndrome should be developed. Repeated acquisition procedures would be an appropriate baseline for the study of learning impairment; procedures for the explicit evaluation of memory impairment would assist in clarifying the nature of any learning impairment observed.

Methanol

Methanol deserves special scrutiny because of the potentially increasing use as a fuel. Methanol produces blindness and life-threatening acidosis in humans following the ingestion of moderately large doses. Although neurotoxicity expressed in the visual system is a hallmark of methanol toxicity, the syndrome remains incompletely characterized. Work with primates has focused on retinal changes; other changes in the visual system or in visual function in nonhuman primates are unknown. Visual evoked potentials and electroretinography are valuable for descriptive purposes; the magnitude of these changes might predict outcome and might also prove to be a useful index of the therapeutic efficacy of methanol antidotes.

□ **Recommendation 16.** Because methanol offers special risks to the visual functions of primates, additional effort should be made to characterize the visual hazards of methanol and their relationship to exposure.

There are a number of clinical reports of damage to the basal ganglia, delayed motor dysfunction resembling parkinsonism, and gross brain injury following methanol exposure (Erlanson et al. 1965; Guggenheim et al. 1971; Aquilonius et al. 1978, 1980; Ley and Gali 1983). This has yet to be studied in primate models of methanol intoxication.

□ **Recommendation 17.** An animal model of methanol-induced motor disorders should be developed. Such a model would assist in determining if acidosis is a necessary precondition for the emergence of methanol-induced motor disorders and if antidotes are effective.

It has been demonstrated that the expression of acidosis is mediated by a folate-dependent metabolic pathway, and that the inhibition of methionine synthetase (produced with nitrous oxide exposure) can provoke methanol-induced acidosis in rats, a normally insensitive species. Furthermore, following a few hours of exposure to nitrous oxide, monkeys treated with 1 g methanol/kg body weight developed acidosis (Eells et al. 1983); this is a much lower dose than is normally required to produce the acute syndrome. This observation might provide a basis for a more sensitive model.

□ **Recommendation 18.** More sensitive animal models of methanol toxicity should be developed, perhaps through the manipulation of folate metabolism. Repeated or continuous exposure to low concentrations of methanol should then be undertaken to determine if acidosis can be produced, and if systemic acidosis is a precondition for the expression of toxicity.

□ **Recommendation 19.** Clinical trials should be undertaken with 4-methylpyrazole, a new drug that is a promising methanol antidote and a candidate for widespread deployment in emergency facilities.

Metals and Inorganic Compounds

Lead (Pb) is the prototypical metallic automotive emission; it is instructive to review our experience with Pb before other metals are added to automotive fuels. The fuel additive tetraethyllead has been responsible for multiple deaths from neurotoxicity and was the focus of a major public health controversy (Rosner and Markowitz 1985). The neurobehavioral toxicity of Pb would most probably have been detected had some systematic approach to testing been in place. Exposure increases the frequency of conditioned behavior of rats at blood levels of Pb below the current clinical definition of elevated Pb burden that requires further diagnostic intervention (Cory-Slechta et al. 1985). Morphological changes in the brain that accompany these functional changes have not been identified.

Another metal, manganese (Mn), however, can produce functional as well as morphological changes. Methylcyclopentadienyl manganese tricarbonyl (MMT) is a potential gasoline additive that has antiknock properties. When used in diesel fuels, Mn additives improve combustion and reduce smoke. The chronic toxicity of the emission product may be subtle, delayed, and readily confused with diseases of other etiology. The syndrome progresses from manic psychosis and disturbances of speech and gait in the early phase, to disturbances of speech, a fixed jovial facial expression, clumsiness and hyperemotionalism in the intermediate stage, and later, muscular hypertonia and tranquil euphoria with memory loss and intact sensory function (Rodier 1955; Mena et al. 1967; Barbeau 1984).

The National Academy of Sciences (1973) reviewed the toxicity of Mn, expressed concern about the Mn emission problem, and made twenty-five research recommendations. Although Mn is not now regulated as a hazardous pollutant, knowledge of the health effects of Mn is limited, especially about the relationship of exposure concentration and duration to effects. The existing data are inadequate to conduct a risk analysis. The risk of adverse health effects from Mn emissions should be characterized as unknown but not necessarily unlikely. The potential injuries are great enough to warrant further study before any

significant increase in exposure is contemplated.

Mn is an essential trace element, but at some higher dose it becomes toxic. The relationship between dose and adverse effect remains unclear, despite the fact that the toxicokinetics of Mn have received fairly detailed attention (Dastur et al. 1971; Newland et al. 1987). The literature provides only limited insight into whether increased exposure increases the severity of effect, as well as the number of individuals affected, or merely shortens the time to toxicity. Children, in particular, may be at high risk, because, as with Pb emissions, (1) the amount of Mn in dust and soil should increase with proximity to the emission source, (2) children put their hands and many other things in their mouths, (3) children become more mobile at 6 months of age, and (4) younger organisms absorb a larger proportion of the administered dose (Cahill et al. 1980; Rehnberg et al. 1981).

Most of the clinical literature consists of effects reported following an idiosyncratic exposure, rather than well-controlled experiments. The emphasis has been on identifying toxicity, and not on generating an orderly dose/effect or dose/response function. There are anecdotal reports of delayed toxicity commencing several years after the termination of exposure (Cook et al. 1974). Changes in human behavior from exposure to Mn at levels that are 20 percent of current recommended exposure limits have been reported by Roels et al. (1985).

□ **Recommendation 20.** Should any increase in Mn emissions be contemplated, an expert committee should be formed to review the neurotoxicity of Mn and the adequacy of current exposure estimates, and to consider the benefits of a chronic study in primates. If significant exposure is contemplated, several experiments must be undertaken to provide data for risk analysis that provide close attention to neurobehavioral function, and consequent regional neurochemical assessment. Early signs of altered dopaminergic function should be examined.

□ **Recommendation 21.** Should any increase in Mn emissions be contemplated, kinetic studies of Mn should be undertaken, with the emphasis on brain uptake and the ingestion of accumulated dust by neonates. Estimates of possible intake should be modeled; exposure of neonatal primates and evaluation of neurobehavioral toxicity should be considered.

Studies of the acute toxicity of MMT have identified seizures and Clara cell necrosis as sequelae to exposure. The delayed effects of acute or chronic low doses have not been well characterized, and are of interest per se; the nervous system is of interest because of the greater access an organic metal should have to the central nervous system and because of the evident neurotoxicity of Mn in primates. Organic complexes or salts might facilitate entry and shorten the time to toxicity.

□ **Recommendation 22.** The acute and chronic neurobehavioral toxicity of MMT should be more adequately characterized.

Conclusions

Determining the acute effects of chemicals on behavior and nervous system function is important to the evaluation of neurotoxicity: acute reversible effects may be the effects of major concern. Similarly, repeated or continuous exposures are needed to characterize toxicity that is delayed or cumulative, to observe the development of tolerance (or reverse tolerance), and to characterize the reversibility of adverse effects. Initial screening for subtle sensory or perceptual impairments, affective disorders, or cognitive and intellectual dysfunction needs to be conducted, and, finally, highly focused studies may be needed to fully characterize hazards using methods that are dictated by the nature of the system or function affected, such as specialized evaluations and refined neuropathological, neurochemical, and neurophysiological techniques.

Since the neurobehavioral toxicity of

many compounds present in automotive emissions is unknown, they should be studied systematically, proceeding from acute, then through repeated administration, experiments and subchronic exposures, and finally to detailed characterizations of injuries and mechanisms. The U.S. Environmental Protection Agency has promulgated guidelines for a variety of neurobehavioral studies, including neuropathology (1985c), motor activity—for example, wheel-running studies (1985b), schedule-controlled (learned) behavior (1985f), and a battery of structured functional observations (1985a). Adopting such tests and incorporating them into a more comprehensive testing strategy is a logical step in developing a rational approach to answering the many unanswered questions surrounding safety evaluation of automotive emissions and their fractions.

Such a testing strategy, depending on a network of methods and techniques, offers several advantages. First, it reduces the likelihood of overlooking a hazard or class of hazards, which any single screening method might miss.

Second, it can be structured in "tiers," in such a way that each tier provides essential information for subsequent tiers as well as useful information in its own right. Such a tiered format is likely to speed up the testing process as well as achieve significant economies in laboratory operations that would serve to offset the potential expense of an extensive screening program. For example, acute toxicity evaluations may serve a dose-ranging function for subsequent repeated-exposure experiments; the findings in both may later give direction to detailed mechanistic studies.

□ **Recommendation 23.** The neurobehavioral toxicity of many constituents present in automotive emissions is unknown and should be studied using a tiered-testing strategy. An expert committee should prioritize the selection of compounds for a systematic testing program, and recommend specialized evaluations when appropriate.

Summary

This chapter has described adverse neurobehavioral effects of automotive emissions and offered research recommendations to facilitate their detailed characterization.

- Neurobehavioral toxicity has not been evaluated thoroughly for most of the compounds that are known to produce adverse effects, nor have screening and evaluation procedures for such toxicity been applied systematically to the universe of chemicals that mobile sources produce.
- To some extent, the absence of data is attributable to a lack of process. Those deficits could be remedied by subscribing to a tiered-testing strategy, with its initial agenda of test substances prescribed by an expert committee. The strategy will provide a disciplined approach, relying on animal studies and supplemented by available literature, for dealing with a large collection of chemicals with largely unknown effects. However, many new tests, procedures, and models need to be developed to assess the adverse health effects that can reasonably be expected to be encountered.
- To another extent, the lack of visibility, prominence, and/or appreciation accorded to neurobehavioral toxicity, even with respect to substances where such effects are well-documented and widespread, contribute to the data shortage. In particular, we should be immediately concerned with:
  - –Sensory irritation and/or repeated and chronic effects that may alter the quality of life;
  - –Effects of CO on complex human performances, and on special populations with exaggerated sensitivity (for example, the fetus, the infant, and the aged);
  - –Effects of petroleum hydrocarbons on behavior, and brain structure and function;
  - –Methanol hazards, should methanol come into wider use; and
  - –Metallic fuel additives, especially Mn, should that come into wider use.

Summary of Research Recommendations

Although the behavioral and neurosciences have already been used to good effect in toxicity evaluation, some areas need further research and development effort, both to demonstrate feasibility and to improve cost-effectiveness. Such a research effort should include attempts to:

- Undertake testing via a tiered approach for chemicals about which little is known (Recommendation 23) before sophisticated and expensive procedures are used to examine candidate toxicants for their capacity to impair learning, memory, cognition, and intellectual functioning (Recommendation 4).
- Develop rapid tests of sensory impairment that can be used in conjunction with functional observation batteries, and develop comprehensive psychophysical studies directed at specific functional impairments, including impairments of complex auditory and visual discriminations (Recommendation 1).
- Develop animal models of affective disorders. Selected reference substances, drugs as well as toxicants, should be used to validate these models before they are used to test uncharacterized chemicals or mixtures (Recommendation 3).

In addition, more specific recommendations for certain automotive emissions can be made immediately. These are outlined here according to priority and contingent upon increases in the level of exposure to a particular emission or deployment of new technology.

HIGH PRIORITY

Recommendations 6, 23	Whole emissions and photochemical by-products deserve immediate attention. Acute and repeated-exposure wheel-running studies should be used to characterize emissions, to differentiate the behavioral consequences of materials that affect primarily the upper or lower airways, and to determine whether the observed effects are attributable to alterations in irritant receptors in lung or lung innervation, frank lung injury, or actual alterations in the peripheral or central nervous systems. The agents responsible for rebound hyperactivity should be determined.
Recommendation 5	Eye irritation is one of the most frequently complained-about effects of automotive emissions and photochemical products. Quantitative procedures should be developed using eye-only exposures to provide estimates of aversiveness derived from behavior under the control of irritant stimulation, and from measurement of blink, heart, and respiratory rates. The development and validation of animal models would be particularly useful.
Recommendation 8	Signs and symptoms of exposure to automotive emission and photochemical by-products, ranging from asthmatic attacks to com-

plaints of breathing difficulties, may be conditioned to occur after exposure to concentrations that evoke no response in naive subjects. Experiments to define the exposure parameters that produce conditioned alterations in sensitivity to airborne irritants are needed.

Recommendation 12 Prenatal exposure to CO can alter the behavior of offspring. Such effects may be of great importance and should be studied further with the techniques of modern developmental neurobiology.

Recommendation 14 The neurobehavioral toxicity of petroleum hydrocarbons in unleaded automotive fuels has received virtually no serious attention. Acute performance impairment should be a major focus of investigation; acute effect determinations are also necessary to ascertain if preventing acute effects will prevent chronic toxicity.

MEDIUM PRIORITY

Recommendation 2 In humans and rodents, the reduction in olfactory sensitivity produced by oxidants and aldehydes should be described as a function of concentration and duration of exposure.

Recommendation 7 A model of human populations with compromised pulmonary function should be developed, because such populations are more sensitive than others to oxidant exposure. Experimental models of chronic obstructive pulmonary disease should be developed that will permit quantitative estimation of exaggerated oxidant sensitivity and display behavioral effects that resemble those produced by chronic exposure to oxidants.

Recommendations 9, 10, 11 The aspects of performance most susceptible to disruption by CO should be identified. The relative importance of different exposure parameters in determining the extent of behavioral impairment should be described. Exaggerated sensitivity in the elderly and those with cardiovascular or respiratory insufficiency should be examined.

Recommendation 13 Repeated-exposure studies utilizing quantitative morphometric neuropathology should be undertaken in experimental animals exposed to petroleum hydrocarbons. Chronic solvent exposure usually does not produce gross central nervous system lesions, but rather a selective wasting syndrome that may not be evident in measurements of gross brain weight.

Recommendation 15 A primate model of the solvent syndrome should be developed. Repeated-acquisition procedures would be an appropriate baseline for the study of learning impairment; procedures for the explicit evaluation of memory impairment would assist in clarifying the nature of any learning impairment observed.

CONTINGENT PRIORITY

Recommendation 16 Because methanol offers special risks to the visual functions of

primates, additional work should be performed to assist in characterizing the hazards and their relationship to exposure.

Recommendation 17 An animal model of methanol-induced motor disorder should be developed. Such a model would assist in determining if acidosis is a necessary precondition for the emergence of methanol-induced motor disorders, and if antidotes are effective.

Recommendation 18 More sensitive animal models of methanol toxicity should be developed, perhaps through the manipulation of folate metabolism. Repeated or continuous exposure of susceptible individuals to low concentrations of methanol should be undertaken to determine if acidosis can be produced, and if systemic acidosis is a precondition for the expression of toxicity.

Recommendation 19 Clinical trials should be undertaken with 4-methylpyrazole, a drug that is a promising methanol antidote, and a new candidate for widespread deployment in emergency facilities.

Recommendation 20 Should any increase in Mn emissions be contemplated, an expert committee should be formed to review the neurotoxicity of Mn and the adequacy of current exposure estimates, and to consider the benefits of a chronic study in primates. If significant exposure is contemplated, several experiments must be undertaken to provide data for risk analysis that provide close attention to neurobehavioral function, and consequent regional neurochemical and neuropathological assessment. Early signs of altered dopaminergic function should be examined.

Recommendation 21 Toxicokinetic studies of Mn should be undertaken, with the emphasis on brain uptake and the ingestion of dust by neonates. Crawling infants ingesting Mn accumulated in dust may be of great concern, as indicated by past experience with Pb. Estimates of possible intake should be modeled; exposure of neonatal primates and evaluation of neurobehavioral toxicity should be considered.

Recommendation 22 The acute neurobehavioral toxicity of any Mn additive should also be evaluated. The delayed effects of single, lower doses have not been well characterized and would be of some interest per se, as might chronic, lower-level exposure. Organic Mn compounds should be very interesting to study in the primate.

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